In the Claims.

Please amend the claims as follows:

1-17.   (Cancelled)

18.   (Previously Presented) A method of PCR analysis comprising the

steps of:

mixing a dsDNA binding dye having a percent saturation of at least 50% with

a sample comprising a target nucleic acid and primers configured for amplifying the target

nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye,

and

monitoring fluorescence of the dsDNA binding dye,

wherein the monitoring step is performed using a fluorimeter having an

excitation range of 450-490 nm and an emission detection range of 510-530 nm, and the dye

has an excitation maximum in a range of 410-465 nm and an emission maximum in a range

of 450-500 nm.

19.   (Original) The method of claim 18 wherein the dye's excitation

maximum is in the range of 430-460 nm and emission maximum is in the range of 450-500

nm.

20.   (Previously Presented) The method of claim 24 wherein the target

nucleic acid comprises a single nucleotide polymorphism, and the identifying step comprises

identifying resultant heteroduplexes and homoduplexes.

21-22. (Cancelled)

23.   (Previously Presented) The method of claim 24 wherein the method

comprises mutation scanning, and the method further comprises

repeating the amplifying and monitoring steps on second sample to obtain a

second melting curve, and

comparing the melting curves.

24.   (Currently Amended) A method of PCR analysis comprising the steps of:

mixing a dsDNA binding dye having a percent saturation of at least 90% 50% with a sample comprising a target nucleic acid and primers configured for amplifying the target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye, and

monitoring fluorescence of the dsDNA binding dye, wherein the monitoring step comprises

melting the amplified target nucleic acid to generate a melting curve, and

identifying the genotype using a shape of the melting curve.

25.   (Currently Amended) The method of claim 24 further comprising the steps of

repeating the mixing, amplifying melting steps with at least one additional target nucleic acid, and

comparing the melting curves.

26.   (Currently Amended) The method of claim 25 further comprising the step of plotting the fluorescence difference between the curves, wherein the melting curve is selected as the standard and is plotted as standard across temperatures and the melting curve for each additional target nucleic acid is plotted as a difference from the standard across the temperatures.

27.   (Original) The method of claim 25 further comprising the step of

temperature shifting the melting curves by superimposing a portion of each curve.

28.   (Currently Amended) The method of claim 27 further comprising the step of plotting the fluorescence difference between the temperature shifted curves, wherein the melting curve is selected as the standard and is plotted as standard across temperatures and the melting curve for each additional target nucleic acid is plotted as a difference from the standard across the temperatures.

29.   (Currently amended) The method of claim 40 wherein the dye is selected from the group consisting of LC Green, Gel Star, PO-PRO™-1, JO-PRO™-1, ~~and BO-PRO™-1,~~ and SYTO® 16.

30.   (Currently Amended) The method of claim 25 wherein the dye is selected from the group consisting of PO-PRO™-1, JO-PRO™-1, BO-PRO™-1, ~~SYTO®-44,~~ SYTO® 45, POPO™-3, SYTO® 12, TOTO™-3, SYTOX® Blue, YOYO®-3, SYTO® 43, SYTO® 11, ~~SYTO®-13, SYTO®-15, BOBO™-3, LO-PRO™-1, SYTO®-23, TO-PRO®-1, SYTO®-20, BOBO™-1, POPO™-1,~~ G5, H5, D6, E6, P6, R6, Y6, Z6, and D8.

31-32. (Cancelled)

33.   (Original) The method of claim 24 wherein the sample further comprises a probe configured to hybridize to the target nucleic acid, said probe labeled with an acceptor dye to accept fluorescent resonance energy transfer from the dsDNA binding dye, and further comprising the step of monitoring fluorescence from the acceptor dye.

34.   (Previously Presented) The method of claim 24 wherein the target nucleic acid is no greater than 100 bp.

35.   (Previously Presented) The method of claim 24 wherein the target nucleic acid is no greater than 61 bp and comprises only a single melting domain.

36.   (Cancelled)

37.   (Previously Presented) The method of claim 25 wherein the target nucleic acid comprises a first melting domain and a second melting domain and the method further comprises the steps of

repeating the mixing, amplifying and melting steps with at least one additional target nucleic acid,

using the second melting domain for temperature axis adjustment, and

comparing the melting curve for the target nucleic acid with the melting curve for the additional target nucleic acid.

38.   (Cancelled)

39.   (Previously Presented) The method of claim 24 wherein the amplifying monitoring, and melting occur in a closed tube, and no reagents are added to the tube subsequent to initiation of amplification.

40. (Previously Presented) The method of claim 39 wherein the monitoring step occurs subsequent to the amplifying step and comprises melting curve analysis.

41-42. (Cancelled)

43. (Previously Presented) The method of claim 39 wherein the monitoring step comprises

generating a melting curve for the target nucleic acid, and

using the melting curve to determine whether the target nucleic acid has the same sequence as a second nucleic acid.

44. (Cancelled)

45. (Currently Amended) A method of PCR analysis comprising the steps of:

providing a mixture of a dsDNA binding dye, a target nucleic acid, and primers configured for amplifying the target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye,

monitoring fluorescence of the dsDNA binding dye,

generating a melting curve for the target nucleic acid,

normalizing magnitude differences of the melting curve,

repeating the providing, amplifying, normalizing, and generating steps with at least one additional target nucleic acid, and

comparing the magnitude-difference-normalized melting curves.

46. (Original) The method of claim 45 further comprising the step of plotting the fluorescence difference between the normalized curves.

47. (Original) The method of claim 45 further comprising the step of

temperature shifting the melting curves by superimposing a portion of each curve.

48. (Original) The method of claim 47 further comprising the step of plotting the fluorescence difference between the temperature shifted curves.

49.   (Currently Amended) A method of PCR analysis comprising the steps of:

mixing a dsDNA binding dye having a percent saturation of at least 50% with a sample comprising a target nucleic acid and primers configured for amplifying the target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye, and

monitoring fluorescence of the dsDNA binding dye, wherein the monitoring step comprises

melting the amplified target nucleic acid to generate a melting curve, and

identifying the genotype using a shape of the melting curve, and The method of claim 24 wherein the dsDNA binding dye is a compound having the formula:
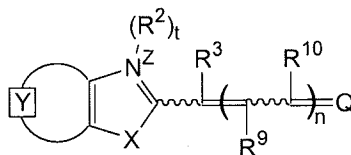
wherein

the moiety $\boxed{Y}$ represents an optionally-substituted fused monocyclic or polycyclic aromatic ring or an optionally-substituted fused monocyclic or polycyclic nitrogen-containing heteroaromatic ring;

X is oxygen, sulfur, selenium, tellurium or a moiety selected from $C(CH_3)_2$ and NR^1, where R^1 is hydrogen or C_{1-6} alkyl;

R^2 is selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, aryl, aryl(C_{1-2} alkyl), hydroxyalkyl, alkoxyalkyl, aminoalkyl, mono and dialkylaminoalkyl, trialkylammoniumalkyl, alkylenecarboxylate, alkylenecarboxamide, alkylenesulfonate, alkylsulfonate, optionally substituted cyclic heteroatom-containing moieties, and optionally substituted acyclic heteroatom-containing moieties;

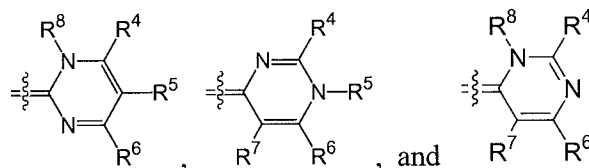t = 0 or 1;

Z is a charge selected from 0 or 1;

R^3 is selected from the group consisting of hydrogen, C_{1-6} alkyl, and -C(O)Ph;

R^9 and R^{10} are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl;

n = 0, 1, or 2;

~~~ indicates a single bond that is in a tautomeric relationship with an adjacent double bond, and

Q is an heterocycle selected from the group of structures consisting of:

wherein R^4, R^5, R^6, R^7, and R^8 are independently selected from the group consisting of hydrogen, halogen, alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, alkenyl, polyalkenyl, alkynyl, polyalkynyl, alkenylalkynyl, aryl, heteroaryl, alkoxy, alkylthio, and dialkylamino, each of which may be optionally substituted; an acyclic heteroatom-containing moiety or a cyclic heteroatom-containing moiety; a BRIDGE-DYE; and a reactive group; each of which optionally includes a quaternary ammonium moiety.

50. (Previously Presented) The method of claim 49 wherein the moiety [Y] represents an optionally-substituted fused monocyclic or polycyclic aromatic ring selected from the group consisting of optionally substituted benzo, optionally substituted pyridino, and optionally substituted naphtho; and X is oxygen or sulfur.
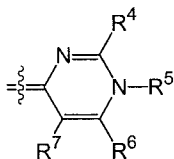
51. (Previously Presented) The method of claim 49 wherein the moiety [Y] represents a benzo or a naphtho having a substituent selected from the group consisting of halo, alkyl, amino, monoalkylamino, dialkylamino, alkylsulfonyl, haloalkylsulfonyl, and optionally substituted phenylsulfonyl.

52. (Cancelled)

53. (Previously Presented) The method of claim 49 wherein R^2 is selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, aryl, aryl(C_{1-2} alkyl), aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, alkylsulfonate, alkylenesulfonate, optionally substituted cyclic heteroatom-containing moieties, and optionally substituted acyclic heteroatom-containing moieties.

54. (Cancelled)

55.   (Previously Presented) The method of claim 49 wherein Q is the
heterocycle:

56.   (Previously Presented) The method of claim 49 wherein R^4, R^5, R^6,
R^7, and R^8 are independently selected from the group consisting of hydrogen, halogen, thiol,
alkylthio, alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl,
trialkylammmoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

57.   (Previously Presented) The method of claim 49 wherein t is 1, n = 0,
and at least one of R^4, R^5, R^6, R^7, and R^8 is selected from the group consisting of halogen,
thiol, alkylthio, C_{2-6} alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl,
trialkylammmoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

58.   (Previously Presented) The method d of claim 57 wherein R^5 is
selected from the group consisting of halogen, thiol, C_{2-6} alkyl, aminoalkyl,
monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammmoniumalkyl, piperidino, piperazino,
4-methylpiperazinium-1-yl, and aryl.

59-60. (Cancelled)

61.   (Previously Presented) The method of claim 57 wherein R^3, R^9, and
R^{10} are each hydrogen; and R^2 is selected from the group consisting of C_{1-6} alkyl, aryl,
aryl(C_{1-2} alkyl), aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl,
trialkylammoniumalkyl, alkylsulfonate, and alkylenesulfonate.

62-64. (Cancelled)

65.   (Previously Presented) The method of claim 43,
wherein the target nucleic acid is a locus of an HLA gene from a first individual and
the second nucleic acid is the same locus of an HLA gene from a second individual.

66.   (Original) The method of claim 65 wherein the melting curve for the
target nucleic acid is similar to the melting curve for the second nucleic acid, and further
comprising the steps of
generating a melting curve for a mixture of the target nucleic acid and the
second nucleic acid, and

comparing the melting curve for the target nucleic acid or the second nucleic acid with the melting curve for the mixture of the target nucleic acid and the second nucleic acid.

67-82.   (Cancelled)

83.   (New) The method of claim 45 further comprising the step of plotting the fluorescence difference between the magnitude difference normalized curves, wherein the melting curve is selected as the standard and is plotted as standard across temperatures and the melting curve for each additional target nucleic acid is plotted as a difference from the standard across the temperatures.

84.   (New) The method of claim 83 wherein the standard is plotted as zero across all temperatures.

85.   (New) The method of claim 47 further comprising the step of plotting the fluorescence difference between the temperature shifted curves, wherein the melting curve is selected as the standard and is plotted as standard across temperatures and the shifted melting curve for each additional target nucleic acid is plotted as a difference from the standard across the temperatures.

86.   (New) A method of PCR analysis comprising the steps of:

providing a mixture of a dsDNA binding dye, a target nucleic acid, and primers configured for amplifying the target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye,

monitoring fluorescence of the dsDNA binding dye,

generating a melting curve for the target nucleic acid,

normalizing the melting curve,

repeating the providing, amplifying, normalizing, and generating steps with at least one additional target nucleic acid, and

comparing the normalized melting curves; wherein no derivative curve of the melting curve is generated.